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(71) Applicant *(for all designated States except US)*: **BY-OCOAT LLC** [US/US]; 320 Northpointe Parkway, Suite D., Acworth, GA 30102 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BURWELL, Steve, R.** [US/US]; 1777 Brookstone Place, Acworth, GA 30101 (US). **BUSCH, Fred** [US/US]; 35 Mohawk Avenue, Clementon, NJ 08021 (US). **RUSSELL, Scott, M.** [US/US]; 1180 Stonebridge Circle, Watkinsville, GA 30677 (US).

(74) Agent: **COMTOIS, Mark, C.**; 1667 K Street, N.W., Suite 700, Washington, DC 20006 (US).

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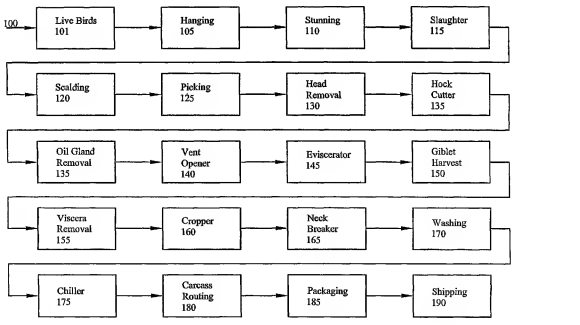

(57) **Abstract:** Disclosed is an antimicrobial solution for treating poultry and meat to substantially eliminate bacteria and microorganism harmful to human. The aqueous solution includes effective amounts of a combination of at least two quaternary ammonium salts, an ammonium halide, trichloromelamine and water. The combination of the quaternary ammonium salt can be selected among cetylpyridinium chloride, N-alkyl dimethyl benzyl ammonium chloride and alkyl dimenthyl ethyl benzyl ammonium chloride.

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ANTIMICROBIAL SOLUTION AND PROCESS

[0001] This application claims the filing-date priority to U.S. Provisional Application No. 60/451,678 filed March 5, 2003, and U.S. Provisional Application No. 60/507,949 filed October 3, 2003, both of the provisional applications are incorporated herein in their entirety.

BACKGROUND

[0002] The present invention generally relates to compositions and methods for eliminating pathogenic microorganisms in food products. More specifically, the present invention is directed to aqueous compositions for treating meat and poultry to substantially eliminate pathogenic bacteria.

[0003] Prevention of food-borne illness has been of paramount concern for the food industry, the public and the regulatory agencies. *Salmonella* is one of the more common intestinal infections with potentially fatal consequences. The U.S. Centers for Disease Control and Prevention reports that every year approximately 40,000 cases of salmonellosis are reported in the United States. Because many milder cases are not diagnosed or reported, the actual number of infections may be thirty or more times greater. Salmonellosis is more common in warmer months than during the winter months. Young children, the elderly, and the immuno-compromised are the most likely to have severe infections. It is estimated that approximately 600 persons die each year with acute salmonellosis.

[0004] Other food-borne microorganisms include *Aeromonas hydrophila*; *Arcobacter butzleri*; *Bacillus cereus*; *Campylobacter jejuni*; *Escherichia coli* ("e-coli"); *Listeria monocytogenes*; *Staphylococcus aureus*. These and other microorganisms adhere to poultry and meat tissues, making removal of the microorganisms difficult with rinsing

alone. Consequently, other treatments including irradiation, chemical treatment and physical processing have been used to address this problem. For example, trisodium phosphate has been used in poultry processing to eliminate *Salmonella*. However, studies have provided conflicting results on efficacy of trisodium phosphate against treating Salmonella.

[0005] U.S. Patent No. 5,366,983 discloses a composition containing an aqueous solution of a quaternary ammonium compound ("QAC"). However, it has been reported that quaternary ammonium cationic surfactants including alkylpyridinium and cetylpyridinium chloride ("CPC") and cetylpyridinium bromide ("CPB") were effective 'in removing *S. typhimurium* and not other types of microorganisms. It has also been found that treatment with CPC requires contacting the meat or poultry with a large quantity of CPC for long periods of time. This requires costly downstream processing steps to remove the QAC and other chemicals in the composition. Typically, this is done by recapturing the product as it is sprayed and hauled out similar to toxic waste

[0006] U.S. Patent No. 5,855,940 discloses a composition containing QAC for inhibiting attachment of and removing pathogenic toxin-producing *Escherichia* contamination. This patent discloses a composition containing QAC selected from the group consisting of alkylpyridinium, tetraalkylammonium and alkylalcyclic ammonium salts in an aqueous solution.

[0007] Other treatment methods include treatment with a chlorine solution or with a solution of tri-sodium phosphate. Chlorine solutions have been found ineffective in eliminating all of the pathogenic microorganisms. Tri-sodium phosphate has been used in reprocessing stage where the inside and the outside of the poultry is sanitized. This process requires filtering the reprocessor's water before disposal in order to remove tri-sodium phosphate.

[0008] Presently, there are no known effective antimicrobial agents that are effective against a broad range of microorganisms. Accordingly, there is a need for a

composition and a method for treating contaminated poultry and meat to eliminate a broad range of microorganisms.

[0009] Therefore, an object of the present invention is to provide a composition and methods for killing food-borne microorganisms. Another object of the invention is to provide a composition for substantially inhibiting growth of microorganisms in poultry and meat tissue. Still another object of the invention is to provide a composition that is safe for human ingestion. These and additional objects, features, and advantages of the present invention will become apparent after reading the following detailed description of the exemplary embodiment of the invention taken in conjunction with the appended drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] The invention is illustrated with the aid of the following non-limiting drawings in which:

Figure 1 is a flow chart showing the conventional processing steps taken in poultry processing.

Figure 2 is a flow chart showing a processing method according to one embodiment of the invention.

Figure 3 shows the comparative effect of an antimicrobial solution on pathogenic and spoilage bacterial isolates as compared with a control solution.

Figure 4 comparatively shows the reduction of bacterial colonies when exposed to an antimicrobial solution according to one embodiment of the invention and a control solution.

Figure 5 shows the comparative effect of an antimicrobial composition according to another embodiment against a control solution.

Figure 6 comparatively shows the reduction of bacterial colonies when exposed to an antimicrobial solution according to one embodiment of the invention and a control solution.

Figure 7 comparatively shows the effects of an antimicrobial solution at various concentrations as compared with a control solution.

Figure 8 comparatively shows the comparative effect of an antimicrobial solution on *Salmonella typhimurium* as compared with a control solution.

Figure 9 comparatively shows the effect of the antimicrobial solution at various concentrations on *Listeria moncytogenes.*

Figure 10 comparatively shows the effect of the antimicrobial solution at various concentrations for eliminating colony forming units of *Listeria moncytogenes.*

Figure 11 comparatively shows the effect of the antimicrobial solution on *E. coli* at various concentrations on *E. coli.*

Figure 12 comparatively shows the effect of the antimicrobial solution at various concentrations for eliminating colony forming units of *E. coli.*

Figure 13 comparatively shows the effect of the antimicrobial solution on *E. coli* at various concentrations on *Staphylococcus aureus.*

Figure 14 comparatively shows the effect of the antimicrobial solution at various concentrations for eliminating colony forming units of *Staphylococcus aureus.*

Figure 15 comparatively shows the effect of the antimicrobial solution at various concentrations on *Pseudomonas fluorescens.*

Figure 16 comparatively shows the effect of the antimicrobial solution at various concentrations on *Shewanella putrefaciens*.

Figure 17 comparatively shows the effect of the antimicrobial solution for eliminating colony forming units of *Campylobacter jejuni* at a dilution of 1:150.

Figure 18 shows the effect of the antimicrobial solution when used to treat various microorganisms attached to food contact surfaces.

Figure 19 compares salmonella content in control samples treated with water and test samples treated with diluted antimicrobial solution.

Figure 20 compares microbial content in control samples treated with water and test samples treated with diluted antimicrobial solution.

DETAILED DESCRIPTION OF THE INVENTION

[0011] In one embodiment, the invention is directed to an antimicrobial composition for treating poultry and meat tissue against food-borne microorganisms. Specifically, an embodiment of the invention is directed to a composition including cetylpyridinium halide, benzalkyl ammonium halide, trichloromelamine and water. The composition can optionally include additional surfactants and other antimicrobial agents.

[0012] The cetylpyridinium halide can be any (or a combination) of cetylpyridinium chloride, cetylpyridinium bromide, Luarel Pyridinium Chloride, Laurel Pyridinium BromideMyristal Pyridinium Chloride or Myristal Pyridinium Bromide.

[0013] Ammonium halide can be any (or a combination) of alkyl dimethyl benzyl ammonium chloride, N-Alkyldimethyl ethyl benzyl ammonium chloride, alkyl trimethyl ammonium chloride, alkyl trimethyl ammonium bromide, alkyl quinolinium ammonium chloride, alkyl quinolinium ammonium bromide, alkyl imidazolinium ammonium

chloride, alkyl imidazolinium ammonium bromide and alkyl dimethyl benzyl ammonium bromide.

[0014] The quaternary ammonium salt can include, among others, alkylpyridinium, tetralkyl ammonium and alkylacicyclic ammonium salts. An exemplary quaternary ammonium salt is AD-16 quaternary ammonium salt.

[0015] In one embodiment, the aqueous composition contains cetylpyridinium chloride in an amount of about 3.5 – 8 wt.% (preferably, 7.5 wt %); N-alkyl dimethyl benzyl ammonium chloride in an amount of about 0.005 – 0.1 wt.% (preferably, 0.01 wt.%); trichloromelamine in an amount of about 0.005 – 0.02 wt.% (preferably, 0.01 wt.%; AD-16 quaternary ammonium salt in an amount of about 0.005 – 0.02 wt.% (preferably, 0.01 wt.%) and a balance of water.

[0016] A composition according to another embodiment of the invention comprises cetylpyridinium chloride in an amount of about 6 – 8 wt.% (preferably, 7.5 wt.%); N-alkyl dimethyl ammonium chloride in an amount of about 0.005 - 0.02 wt.% (preferably, 0.01 wt.%); trichloromelamine in an amount of about 0.005 - 0.02% (preferably, 0.01 wt.%); AD-16 quaternary ammonium salt in an amount of about 0.005 - 0.02% (preferably, 0.01 wt.%) and a balance of water.

[0017] In a preferred embodiment of the invention, an antimicrobial composition comprises Cetylpyridinium Chloride (7.5 parts); Alkyl dimethyl benzyl ammonium chloride (0.1 part); Trchloromelamine (0.1 part); Arquad AD-16 (trimethyl ammonium chloride) (0.1 part); Water (92.2 parts).

[0018] As will be discussed below, Applicants' experiments show that the preferred embodiments are equally effective even when diluted with water up to a certain point. For example, it has been found that the active ingredients (anything except water) can be diluted with water in the range of 50-400 part water to one part active ingredient and still perform effectively.

[0019] Moreover, the antimicrobial solution has been found effective for applications other than treatment of poultry. For example, a composition according to the above-identified solution has been found to effective for treating poultry litter. The proposed antimicrobial solution is added to the poultry litter as it is being created at the paper mill. It is applied by electrostatic sprayers while on the paper processing lines. It is applied to both sides of the paper prior to being chopped into the proper size for use as poultry litter.

[0020] Another suitable application of the antimicrobial compositions disclosed herein is in production of raisins. In a typical raisin operation plant, grapes are laid out on a substrate in open air to expose the grapes to the ambient air for drying. The substrate, also being exposed to the ambient air, is contaminated with various airborne microorganisms. Upon continual use of the substrate, the substrate will contaminate the grapes. Applicants have discovered that treating the substrate with an antimicrobial solution as disclosed herein will reduce, if not eliminate, the cross-contamination problem. In a raisin operation plant, the grapes will lay on a paper substrate in the fields near the location of the vines until they dry into raisins. Mold and mildew begins to grow as moisture develops from changing dew point caused by weather. The antimicrobial solution disclosed herein prevents the growth of the mold and mildew as it is used as a sealant in the paper. The antimicrobial solution is applied by spraying or dipping.

[0021] In addition to the above active ingredients, the composition may include surfactants, fillers and other additives specifically designed to affect the viscosity, thixotropy or ability of the aqueous solution to adhere to and penetrate tissue. Suitable exemplary additives include surfactants such as Triton X-100 for better cell penetration.

[0022] Figure 1 is a flow chart showing the conventional processing steps taken in poultry processing. With reference to Figure 1, conveyor 100 is used to transport the poultry through various steps of the processing plant. At step 101 live birds brought in are loaded onto an automated conveyor belt at step 105. At step 110, live birds are

exposed to electrical current; this stage is also known as stunning. The birds are stunned when their heads (primarily the comb) contact a saline solution in the bottom of the stunner through which an electrical current is surging. This jolt of electricity is not severe enough to permanently damage or kill the bird, but immobilize the bird and allow the body of the bird to become relaxed enough to allow for automated killing. With the birds still hanging upside down, and necks outstretched due to stunning, the birds are exsanguinated by an automated circular blade at step 115 of the process.

[0023] After the blood is removed from the poultry, at step 120, the bird is submerged in a large tank of circulating hot water (128-134° F) for about 2 minutes to loosen the feathers. This process is called "scalding". The feathers and skin of the bird come out of the scalding process saturated with water. This process is particularly susceptible to bacterial cross-contamination since the birds are immersed in a common bath. Next is the picking process 125, and head removal 130 are performed. The birds are then dropped off of the aerial conveyor system at hock cutter step 135.

[0024] The U.S. Department of Agriculture ("USDA") requires one quart of fresh water or recycled water to be added for each bird that enters the scald tank; thus, there is a continuous overflow of water from the scald tank. In one embodiment of the invention (See Figure 2), the scald tank is replenished with the rinsate from the spray system downstream with the antimicrobial solution at slightly less than full strength (e.g., 502 ppm) in order to decrease the cross-contamination of pathogenic bacteria in the scald tank. At start up each day, it will be necessary to treat the scald tank after it's initially filled with fresh water with the antimicrobial solution at full strength. This will assure treatment of birds that pass through the scald tank, prior to the spray system rinsate recycle process.

[0025] Referring once again to Figure 1, at step 135 preen gland is removed and at step 140 a venting machine cut around the vent or the anus of the bird, removing about two inches of any possible remaining fecal mater from the colon. A chlorinated water

spray is utilized on this machine to keep any possible fecal material from contaminating the outside skin of the bird. The next machine is the eviscerator (step 145). It uses a spoon-like device to pull the internal organs out of the body cavity. This machine typically has a chlorinated water spray to keep any intestinal contents from coming into contact with the outside surface of the bird. This machine does not entirely remove the guts or "viscera" from the carcass, but gently drapes the "viscera package" onto the back of the bird where it can be viewed by USDA inspection personnel for possible diseases. After the USDA inspector has viewed the entire bird, including the viscera package, the viscera are removed from the carcass and fall into the same offal trough which has already received the preen gland, head, and neck.

[0026] In some plants, the gizzard, heart, and liver are harvested from the birds for human consumption (step 150). However, the majority of processors now just let these become part of the inedible material leaving the plant because they receive more money for those products in the animal feeds business than in the consumer market. After the viscera are dropped into the trough or "offal line" (step 155), the lungs are suctioned out of the body cavity and then enter the offal line. This fully eviscerated carcass hanging on the shackle line by the legs is commonly referred to as the WOG (whole carcass without giblets). The next two steps are cropper 160 and the neck breaker 165.

[0027] After USDA inspection and viscera removal, it is necessary to thoroughly wash the inside and outside of the carcass. While the carcasses are still moving on an overhead conveyor system, they pass through at least one "inside/outside bird washer". This system is comprised of a stainless steel cabinet that is designed for automated washing of carcasses. Several gallons of water are used to clean each individual carcass – inside and out. All of the water used in these wash cabinets is directed to the offal line. Thus, the spent wash water, water which is continually used to rinse off the evisceration machinery, water from hand and knife washing stations, and fresh water as needed, is utilized to move the inedible material through the offal troughs and is deposited into the waste stream.

[0028] Figure 2 is a flow chart showing a processing method according to one embodiment of the invention. With reference to Figure 2, an antimicrobial solution is applied to the poultry at stage 170. This application is typically done by spraying the suspended poultry. The spraying process can include the outside as well as the insides of the poultry. During the spraying process, a predetermined amount of the antimicrobial solution is sprayed on the carcass. As shown in Figure 2, the runoffs are then collected and supplied to the scalder for reuse; thus, the antimicrobial additive along with fresh water is provided counter-current to the direction of the carcass. Thereafter, they may be reused in the scalder or added to the waste stream. If necessary, additional antimicrobial solution can be added to the recycled stream 200 in order to bring the concentration to the desired level. While the concentration may be varied depending on the application, it has been found that a concentration of about 300 – 600 PPM of antimicrobial solution to water can be effective.

[0029] In another embodiment, the process includes a first exposure of the poultry to the antimicrobial solution in the scalder. Filtered rinse-water from the antimicrobial spray positioned just prior to the chiller can be added to the fresh water entering the scalder at a concentration of about 450-600 ppm (except for start-up where the initial scald tank water will be activated with antimicrobial solution at full strength.) This water can then pass over the carcasses and exit the scalder at the overflow (where carcasses enter the scalder). Thus, during the scalding step, the carcasses will be exposed to a maximum of 450-600 ppm of the antimicrobial solution. The carcasses can then continue down the processing line and through evisceration, cropping, and inside/outside bird washing, and finally pass through the spray cabinet, where a desired concentration of the antimicrobial solution can be applied again. The birds will then pass through the spray cabinet at normal line speed for application of the antimicrobial solution (*e.g.*, about 0.2 gram of the antimicrobial solution per pound of carcass). Testing conducted by an independent laboratory showed that less than about 30 ppm of the antimicrobial solution remains on the carcass after both exposure points. That is, the majority of the

antimicrobial solution drains out of the cabinet, is filtered, goes into the scalder, passes by the carcasses in the scalder and is sent to the waste stream. Material balance calculations demonstrate that approximately 99.9% of the antimicrobial solution will be sent to the waste stream.

[0030] In still another embodiment, A drip tray can be included as part of the application system. As the birds exit the spray cabinet on their way to the chiller tank, they will pass over this drip tray, which will collect any antimicrobial solution containing fluid that drips from the wet carcasses. This tray will extend for the distance covered by the carcasses in the first minute after they exit the spray cabinet, or typically about one-half the distance to the chiller. The liquid that drips into this tray can be combined with the fluid that drains from the antimicrobial spray cabinet and can be recycled back to the scalder. For the remainder of the distance to the chiller (*i.e.*, the second minute of travel time from the spray cabinet), any liquid that drips from the carcasses can go into the plant's existing floor offal collection system and ultimately will be collected as part of the offal.

[0031] As indicated above, after treatment with antimicrobial solution, the carcasses can move via the overhead line to the chilling phase of the process. They drop automatically from the shackle line into a huge tank of water called the pre-chiller. This tank of water is typically held at 55°F and the carcasses remain in the pre-chiller for about 15 minutes. During this time, the carcasses absorb 4 to 5% added moisture. The water in the pre-chiller can be actively aerated to aid in water movement for increased chilling potential and water absorption. This aeration process, combined with the large amount of fat that is present in the pre-chill water, forms a flocculent material that floats on the top of the chill water. This material, typically called "chiller skimmings", is continuously removed from the pre-chiller water and diverted to the offal trough.

[0032] From the pre-chiller tank, the carcasses move into the chiller tank (shown as step 175 at Figure 1). This tank is larger and colder than the pre-chiller, usually 32 -

34°F. The carcasses stay in this tank for about 45 minutes, increasing their moisture content by an additional 3 to 4% in the chiller. USDA allows poultry carcasses to gain a total of 8% added moisture. Constant aeration of the water, combined with the fat that is present in the chiller water, forms a large amount of chiller skimmings. As is the case in the pre-chiller, this material is diverted to the offal trough. After chilling, the carcasses are rehung on a different shackle line for transport to other areas of the plant. They may move to a whole carcass packaging station (step 185), to cut-up or de-boning, or they may be shipped to a different plant for further processing and cooking (step 190).

[0033] The waste streams for antimicrobial solution in the poultry-processing environment are explained in detail below. As stated, the great majority of the antimicrobial solution present in the spray solution goes to the scalder and, after passing through the scalder, is conveyed to the waste stream and the offal. To achieve the desired concentration, additional antimicrobial solution may be added to the rinsate collected from the spray cabinet, prior to introduction into the scalder. Based upon calculations, the maximum concentration of antimicrobial solution that may enter the environment as a result of its intended use will be limited to the amount that remains in the water or combined with organic material after passing through the scalder and any residual that may drip from carcasses after spraying or be rinsed from the carcasses during chilling. (This amount has been calculated to be approximately 502 ppm of the antimicrobial solution residue on the carcass).

[0034] In one embodiment of the invention, the antimicrobial solution is applied by means of electrostatic coating. This can be done after the reprocessin stage or in place thereof. Electrostatic spraying can be done by using air-atomizing induction charge nozzle which allows air and liquid to enter the nozzle separately. The air moves at a high speed through the nozzle and intersects the liquid at the nozzle tip, causing the formation of spray droplets. The droplets are generally 30-40 microns in diameter. The air pressure required is 30-40 PSI, while the liquid pressure is below 15 PSI. As the spray is atomized, the droplets pass a unique embedded induction electrode that induces a charge

on each droplet. A rechargeable battery provides the electrical charge. The negatively charged droplets are propelled onto the target surfaces by the force of the turbulent air stream. The target surface (the poultry) has a naturally positive charge. The electrostatic charge on the spray droplets is negative. Positive electrical charges on the target surface pull the spray droplets to the tops, bottoms and sides of the surface providing 360 degree wrap-around coverage. Once the liquid is shut-off, the air pressure siphons out any remaining spray. Air keeps the nozzle passages clear, reducing maintenance.

[0035] As compared with the conventional treatment methods, Applicants' invention has been found particularly advantageous in that the treatment process is substantially faster and less caustic. In addition, because a smaller amount of antibacterial solution is used, the process is more effective. The following non-limiting examples, further illustrate advantages of Applicants' invention over the conventional antimicrobial solutions and processes. The results in each case illustrate the comparative and unexpected superiority of Applicants' composition.

EXAMPLE 1

[0036] The effects of an antimicrobial solution according to the principles of the invention were studied on pathogenic, indicator and spoilage populations of bacteria associated with broiler chicken carcasses. Scalder water was collected from the overflow end (the entrance end) of a commercial poultry scalder. The water was autoclaved to eliminate all populations of bacteria and bacterial spores to avoid interference during the study. The autoclaved scalder water was evaluated chemically and compared to raw scalder water to ensure that the organic material in raw and autoclaved scalder water were similar.

[0037] A test solution (interchangeably referred to as the antimicrobial solution) was prepared containing cetylpyridinium chloride (7.5 parts), alkyl dimethyl benzyl ammonium chloride (0.1 part), trichloromelamine (0.1) part, Arquad AD-16 (trimethyl

ammonium chloride) (0.1 part) and Water (92.2 parts). Next, a control solution was prepared by admixing CPC (7.5 wt.%) and water (92.5 wt.%). The same solutions were used in all of the examples.

[0038] Sets of test tubes were prepared by adding 9 mL of autoclaved (sterilized) scalder water to sterile polystyrene test tubes. One set was prepared as controls by adding 9 mL of autoclaved scalder water to tubes. Another set was prepared by adding 9 mL of autoclaved scalder water and 1 mL of the test solution as identified above. The pathogens were *salmonella typgimurium ("ST")*, *Listeria monocytogenes ("LM")*, *staphylococcus aureau ("SA")*. The indicator was *Escherichia coli* ("EC") and the spoilage bacteria were *pseudomonas fluorscens ("PF")* and *shewanella putrefaciens ("SP")* were grown overnight in Brian Heart infusion broth at 25°C for 24 hours. Each bacterium was exposed to each autoclaved scalder water-sanitizer combination for 2 minutes to mimic scalding. After exposure period, 1 mL of the suspension was placed into 9 mL of Brian Heart infusion broth and vortexed. One mL of this mixture was placed into the Bactometer module and bacterial growth was measured. The results are provided at Figs. 3-6.

[0039] It can be seen from Figure 3 that the antimicrobial test solution according to Applicants' invention was effective for reducing populations of *Salmonella, Listeria, staphylococcus* and *shewanella* when used in combination with scalder water applications. In the mean time, a substantial reduction is seen for *Escherichia coli* and *Pseudomonas fluorescens*. In comparison, the control solution eliminated much less of any of the above microorganisms.

[0040] Figure 4 comparatively shows the reduction of bacterial colonies when exposed to a test solution and a CPC solution. The colony forming units for *salmonella typgimurium*, *Listeria monocytogenes*, *staphylococcus aureau* and *Escherichia coli* were tested. Although not depicted with Log_{10} CFU in Figure 4, *Pseudomonas* was also reduced to below 10 CFU/mL.

[0041] Figure 5 shows the effect of the test solution as compared with the control solution. It can be seen from Figure 5 that over a period of 24 hours, *Salmonella Typgimurium*, *Listeria Monocytogenes*, *Staphylococcus Aureus* and *Shewanella Putrefaciens* were completely eliminated while *E. coli* and *Pseudomonas Fluorscens* were substantially reduced as compared with samples treated with the control solution.

[0042] Figure 6 comparatively shows the reduction of bacterial colonies when exposed to the test solution and the control solution. Figure 6 is similar to Figure 4 and shows that the colony forming units for all microorganisms where nearly eliminated upon treatment with the antimicrobial test solution. Thus, the antimicrobial solution was extremely effective in eliminating all pathogenic, indicator, and spoilage bacteria tested in combination with scalder water applications. This data also indicates effectiveness of the test solution against very high concentrations of bacteria.

EXAMPLE 2

[0043] Example 2 was conducted to measure the effects of antimicrobial solution at various concentrations on pathogenic, indicator and spoilage populations of bacteria associated with poultry. To this end, scalder water was collected from the overflow end (entrance end) of a commercial poultry scalder. The water was autoclaved to eliminate all populations of bacterial and bacterial spores to avoid interference during the study. The autoclaved scalder water was evaluated chemically and compared to raw scalder water to ensure that the organic material demand in raw and autoclaved scalder water were similar.

[0044] a) Antimicrobial Solution - An antimicrobial solution was as in Example 1 and dilute with deionized water to ratios of about 1:100, 1:150, 1:200, 1:300 and 1:400.

[0045] b) Control solution - Sets of test tubes were prepared as controls by adding 9 mL of autoclaved (sterilized) scalder water to sterile polystyrene test tubes. One set was prepared as controls by adding 9 mL of autoclaved scalder water to test tubes. One set

was prepared by adding 9 mL of autoclaved sclader water and 1 mL of each antimicrobial solution. The control solution, as with the previous examples, comprised a CPC solution in water.

[0046] c) Materials and methods – The pathogens *Salmonella Typhimurium, Listeria Monocytogenes, Staphylococcus Aureus*, the indicator *Escherichia coli* and the spoilage bacteria *Pseudomonas fluorescens* and *Shewanella* were grown overnight in Brian Heart infusion broth at 25 °C for 24 hours. Each bacterium was exposed to each autoclaved scalder water-sanitized combination for 2 minutes to mimic scalding. After exposure period, one (1) mL of the suspension was placed into 9 mL of the Brian Heart infusion broth and vortexed. One (1) mL of this mixture was then placed into the Bactometer module well and bacterial growth was measured. The results are presented at Figs. 7-17.

[0047] d) Results – The antimicrobial test solution disclosed above was found effective for eliminating populations of *Salmonella, Pseudomonas* and *Shewanella* especially when used at concentrations of 1:150 or lower with scalder water applications. Figure 7 comparatively shows the effects antimicrobial solution at various concentrations as compared with a control solution. It can be seen from Figure 7 that bacterial elimination is fairly high for a solution diluted to about 1:100. Figure 8 shows the comparative effect of the test solution on *Salmonella Typhimurium* as compared with a control solution. It can be seen in Figure 8 that the test solution diluted to about 1:100 and 1:150 is very effective in reducing colony forming units. The effect of the antimicrobial solution on *Listeria* is shown in Figs. 9 and 10. With reference to Figure 9, it can be seen that the test solution according to the exemplary embodiment of the invention completely eliminated populations of *Listeria* and *Staphylococcus* at all concentrations, including solutions diluted with water to about 1:400.

[0048] Figure 10 shows that colony forming units were substantially eliminated by the antimicrobial solution at all concentrations. Figures 11 and 12 show the comparative effects of various dilutions of the antimicrobial test solution on *E. coli.* As shown, the

test solution was able to eliminate populations of *E. coli* at a dilution of about 1:100. At dilutions of about 1:150 (or lower) the test solution was able to eliminate all species tested with the exception of *E. coli*. Because *E. coli* is not a pathogen, it is not necessary that it be eliminated at the scalder. Instead, it can be eliminated later in the process. For this reason, a water dilution of about 1:150 has been found to be suitable for the scalder. Figures 13 and 14 show the comparative effects of the test solution at different concentration on *Staphylococcus aureus*. These results are self-explanatory. Figures 15 and 16 comparatively show the effect of the test solution at different concentrations on *Pseudomonas fluorescens* and *Shewanella putrefaciens*. Finally, Figure 17 comparatively shows the effect of the antimicrobial solution for eliminating colony forming units of *Campylobacter jejuni* at a dilution of 1:150. These results verify that the antimicrobial test solution, according to the principles of the invention, is superior over the conventional compositions for treating known microorganisms.

EXAMPLE 3

[0049] The effects of the antimicrobial test solution on pathogenic indicator and spoilage populations of bacteria associated with broiler chicken carcasses attached to food contact surfaces were studied.

[0050] a) Film attachment procedure – The pathogens, *Salmonella Typhimurium, Listeria monocytogenes, Staphylococcus aureus,* the indictor *Escherichia coli* and the spoilage bacterial *Pseudomonas Fluorescens* and *Shewanella putrefaciens* were grown overnight in Brian Heart infusion broth at 25 °C for 24 hours. Five sterile Teflon coupons were coated with 0.200 mL of each of the pathogens, the indicator or the spoilage species of bacteria (total of 30 coupons.) The bacterial inocula were allowed to dry on the surface of the coupon for 4 hours. Each coupon was sprayed for 10 seconds (3 separate sprays) using a 1:100 concentration of the test solution. Each coupon was completely coated with 30 mL solution of this solution. No sanitizer residual or wet appearance occurred. After the exposure period each coupon was rinsed in 100 mL of sterile 1%

buffered peptone broth. One (1) mL of this mixture was then placed into 9 mL of Brian Heart infusion broth and then 1 mL of this mixture was placed into the Bactometer module well for measuring bacterial growth.

[0051] b) Method – A control solution as disclosed above was prepared. In addition, an antimicrobial solution comprising was prepared for testing purposes. A sample of the coupons coated with the control solution and the balance was coated with the antimicrobial test solution. In both applications, electrostatic coating technique was used to adherently coat the entire surface of the coupon substrate.

[0052] c) Results – The results are shown at Figure 18. It can be seen from Figure 18 that the test solution was extremely effective in eliminating populations of *Salmonella, Listeria, Staphylococcus, E. coli* and *Pseudomonas* on food-contact surfaces. Clearly, this method is effective for treating and sanitizing food-contact surfaces before or after processing operation.

EXAMPLE 4

[0053] The effect of the antimicrobial composition which was applied using a sprayer and immersion in treated scalder water on *Salmonella typhimurium* and *E. Coli* attached to broiler carcasses were studied. For this experiment, poultry samples were selected prior to the scalder step of the process. The control samples were treated with water and the test samples were treated with the antimicrobial solution. All samples were treated with salmonella to establish a baseline. Next, two different scalder baths were prepared; one contained scalder water and the other contained scalder water treated with the antimicrobial solution. The control samples were sprayed with water to simulate the washing step 170 (Figure 1). The test samples were processed in the same manner except the scalder water contained the antimicrobial solution and the sprayer contained the antimicrobial solution at a 1:150 dilution. The test was repeated three times (Reps. 1-3) and the salmonella content of the samples were recorded. Figure 19 compares salmonella

content in control samples treated with water and test samples treated with diluted antimicrobial solution.

[0054] The procedure outlined about was repeated except that the samples were treated with *E. Coli* and *Coliform* for establishing a baseline. Here, only one set of control and test samples were tested and the result is presented in Figure 20. Referring to Figure 20, it can be readily seen that *E. Coli* and *Coliform* colony forming units were substantially reduced in test samples as compared with the control samples.

EXAMPLE 5

[0055] The following studies were conducted to evaluate the amount of residual antimicrobial composition left on the broiler chicken carcass after simulated treatment.

[0056] Study I (in-line reprocessing simulation) - Four broiler chicken carcasses were purchased from a local retail outlet. Two of the carcasses were rinsed with water for 3-5 seconds to simulate rinsing that takes place in the processing plant immediately prior to automated in-line reprocessing. The carcasses were then sprayed (to simulate delusion using an in-line sprayer) in an antimicrobial solution prepared according to Example 1 at a dilution of 150:1. The carcasses were allowed to remain for two minutes to simulate the drip time after in-line reprocessing and chilling. The carcasses were then placed into chilled water for 60 minutes to simulate chilling. During the chilling process, the carcasses were periodically stirred to simulate aeration. Additionally, the water was completely exchanged with fresh water after 30 minutes to simulate commercial situations. The carcasses where then cooked at 350 °F for about 45 minutes.

[0057] Study II (In-line reprocessing and scalding simulation) - Two of the carcasses were dipped for two minutes into 130°F water containing an antimicrobial solution prepared according to Example 1 at a 150:1 dilution to simulate commercial scalding conditions. Carcasses were rinsed for 3-5 seconds to simulate the rinse spray

between the scalder and the in-line reprocessing system. The carcasses were then sprayed (to simulate delusion using an in-line sprayer) in the antimicrobial solution. The carcasses were allowed to remain for two minutes to simulate the drip time after in-line reprocessing and chilling. The carcasses were then placed into chilled water for 60 minutes to simulate chilling. During the chilling process, the carcasses were periodically stirred to simulate aeration. Additionally, the water was completely exchanged with fresh water after 30 minutes to simulate commercial situations. Carcasses were then cooked at 350°F for 45 minutes.

[0058] After cooking, the following steps were followed for each study. Fifty grams of skin was collected from each carcass. The skin samples were individually placed into a blender and 200 mL of deionized water was added. The skin was blended on high for 8 minutes. 300 mL of fresh deionized water was added to the blended mixture and blended for an additional 5 minutes. 150 mL of the blended mixture was placed into a sample jar and sent to an independent laboratory for testing and evaluation.

[0059] Independent laboratory evaluation on fully cooked chickens treated with the antimicrobial solution at a 150:1 concentration indicated that a maximum of only 0.02 ppb could be recovered from the skin samples. The residual discovered on the two carcasses in Study I (in-line reprocessing simulation) was 0.02 ppb and 0.02 ppb. The residual discovered on the two carcasses in Study II (in-line reprocessing and scalding simulation) was 0.01 ppb and 0.01 ppb. Because the amount of residual antimicrobial solution recovered from carcasses treated using simulated commercial conditions for in-line reprocessing and scalding and in-line reprocessing was so inconsequential, it was concluded that use of the proposed composition under these conditions would pose no health hazard.

[0060] Independent testing performed on chickens being treated with the antimicrobial solution prior to their submergence in the chiller process for 45 – 60 minutes proved to be very successful in substantially reducing the numbers of pathogens

on the chickens. Microbial testing done prior to the chickens introduction into the chiller water compared to microbial test results after exit from the chiller was in excess of 1 log reductions of the pathogen levels. In other words, the antimicrobial solution can be added to the chiller as it is readily soluble in cold water as well as in warm or hot water.

[0061] These examples illustrate the superiority of Applicants' invention over the conventional CPC compositions. In one aspect, the combination of the various components in the antimicrobial solution work synergistically to bring about a more efficacious compound. As a result, a much smaller percentage of CPC comes into contact with the poultry while far superior bacterial elimination is obtained. Moreover, the conventional composition of CPC is less effective against gram negative bacteria. The antimicrobial solutions disclosed herein have been found to have superior efficacy against gram negative bacteria.

[0062] Although the exemplary embodiments provided herein are directed to a poultry processing line, it will be understood that the disclosed invention can be applied to meat treatment in general without departing from the spirit of the invention.

[0063] It will also be understood by those of skill in the art that although the components of the exemplary embodiments are represented in their respective weight percent, the ratios may nonetheless be varied to include molar or volume percent of each component.

What is claimed is:

1. In a composition for treating microorganisms and other food-borne bacteria, the improvement including an aqueous solution comprising cetylpyridinium chloride in an amount of about 6 – 8 wt.%; N-alkyl dimethyl ammonium chloride in an amount of about 0.005 - 0.02 wt.%; trichloromelamine in an amount of about 0.005 - 0.02 wt.%; AD-16 quaternary ammonium salt in an amount of about 0.005 - 0.02 wt.%; and a balance of water.

2. The composition of claim 1, wherein cetylpyridinium chloride is present in an amount of about 7.5 wt.%.

3. The composition if claim 1, wherein the N-alkyl dimethyl ammonium chloride is present in an amount of about 0.01 wt.%.

4. The composition of claim 1, wherein the trichloromelamine is present in an amount of about 0.01 wt.%.

5. The composition of claim 1, wherein the AD-16 quaternary ammonium salt is present in an amount of about 0.01 wt.%.

6. The composition of claim 1, wherein the water is present in an amount of about 92.47 wt.%.

7. In a composition for substantially eliminating microorganisms and other food-born bacteria, the improvement including an antimicrobial solution comprising cetylpyridinium chloride in an amount of about 6 – 8 wt.%; didecyl dimethyl ammonium chloride in an amount of about 0.005 - 0.02 wt.%; trichloromelamine in an amount of

about 0.005 - 0.02 wt.%; AD-16 quaternary ammonium salt in an amount of about 0.005 - 0.02 wt.%; and a balance of water.

8. The composition of claim 7, wherein cetylpyridinium chloride is present in an amount of about 7.5 wt.%.

9. The composition of claim 7, wherein didecyl dimethyl ammonium chloride is present in an amount of about 0.01 wt.%

10. An aqueous composition comprising effective amounts of a combination of at least two quaternary ammonium salts, an ammonium halide, trichloromelamine and water.

11. The aqueous composition of claim 10, wherein the combination of at least two quaternary ammonium salt is selected from the group consisting essentially of cetylpyridinium chloride, N-Alkyl dimethyl benzyl ammonium chloride and Alkyl dimethyl ethyl benzyl ammonium chloride.

12. The aqueous composition of claim 10, wherein the combination of at least two quaternary ammonium salt is present in an amount of about 6.02 – 8.02 wt.%.

13. In a process for substantially eliminating food-borne microorganisms in poultry by processing the poultry carcass through a scalder for heating the carcass with water and/or steam, a spray station for spraying one or more antimicrobial solutions on the carcass and a chilling station, the improvement comprising applying a composition having of at least two quaternary ammonium salts in combination with trichloromelamine and water at the spray station and recycling said composition for use in the scalder.

14. The process improvement of claim 13, wherein the at least two quaternary ammonium salt is selected from the group consisting of cetylpyridinium chloride, N-Alkyl dimethyl benzyl ammonium chloride and alky dimethyl ethyl benzyl ammonium chloride.

15. The process improvement of claim 13, wherein the combination of at least two quaternary ammonium salt is present in an amount of about 6.02 – 8.02 wt.%.

16. A method for removing food-borne pathogens comprising treating a meat product with an effective amount of a solution consisting essentially of cetylpyridinium chloride, N-alkyl dimethyl benzyl ammonium chloride, trichloromelamine, AD-16 quaternary ammonium salt and water.

17. The method of claim 16, wherein each of cetylpyridinium chloride, N-alkyl dimethyl ammonium chloride, trichloromelamine, AD-16 quaternary ammonium salt is present in an amount of about 6 – 8 wt.%, 0.005 - 0.02 wt.%, 0.005 - 0.02 wt.% and 0.005 - 0.02 wt.%, respectively.

18. The method of claim 16, wherein food-borne contaminants include microorganisms, bacteria and microbial agents.

19. A method for removing microorganisms comprising treating a meat product with an effective amount of a solution consisting essentially of cetylpyridinium chloride, Alkyl dimethyl ethyl benzyl ammonium chloride, trichloromelamine, AD-16 quaternary ammonium salt and water.

20. The method of claim 19, wherein each of cetylpyridinium chloride, didecyl dimethyl ammonium chloride, trichloromelamine, AD-16 quaternary ammonium salt is present in an amount of about 6 – 8 wt.%, 0.005 - 0.02 wt.%, 0.005 - 0.02 wt.% and 0.005 - 0.02 wt.%, respectively.

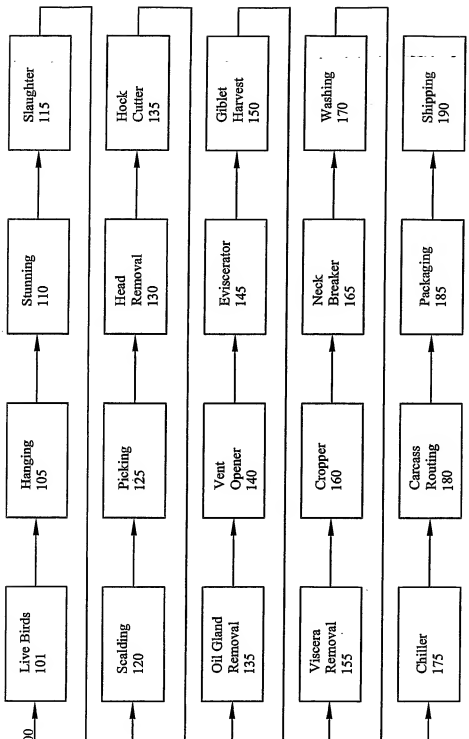


FIGURE 1
(PRIOR ART)

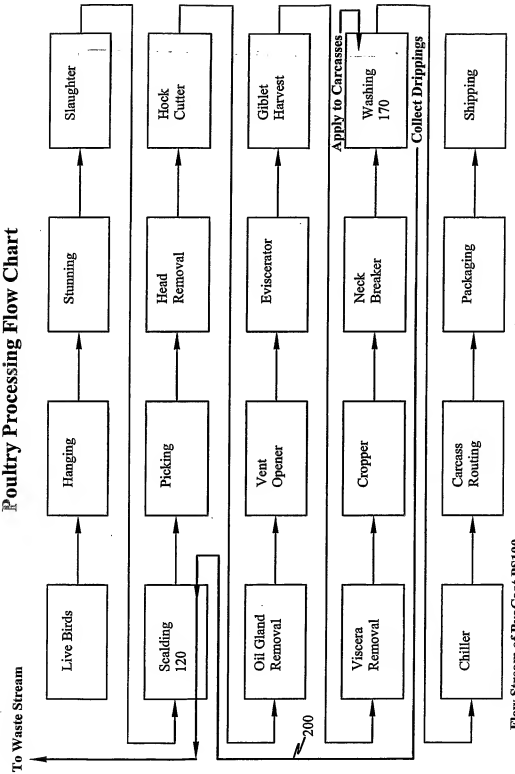
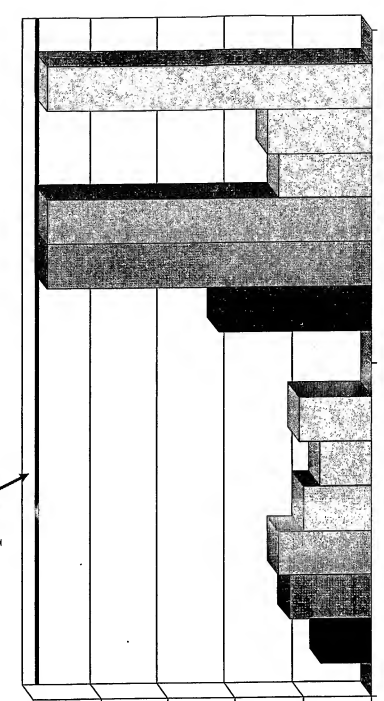

Poultry Processing Flow Chart
To Waste Stream
Live Birds
Hanging
Stunning
Slaughter
Scalding 120
Picking
Head Removal
Hook Cutter
Oil Gland Removal
Vent Opener
Eviscerator
Giblet Harvest
200
Viscera Removal
Cropper
Neck Breaker
Apply to Carcasses
Washing 170
Collect Drippings
Chiller
Carcass Routing
Packaging
Shipping
Flow Stream of ByoCoat PS100

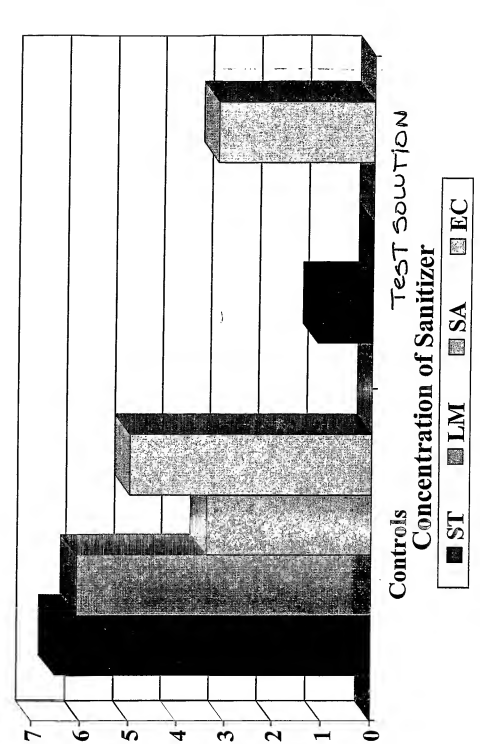
FIGURE 2

Figure 3



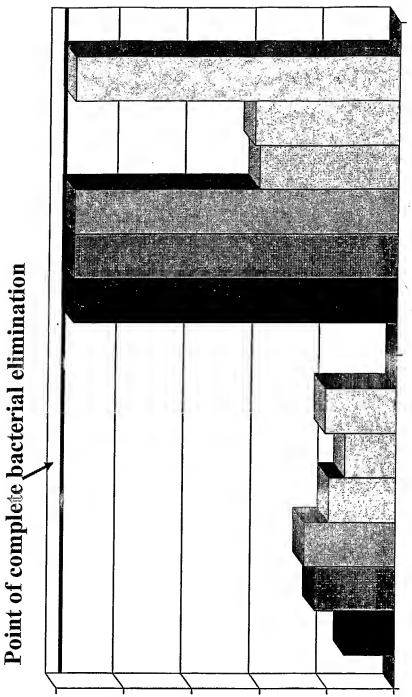
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 4



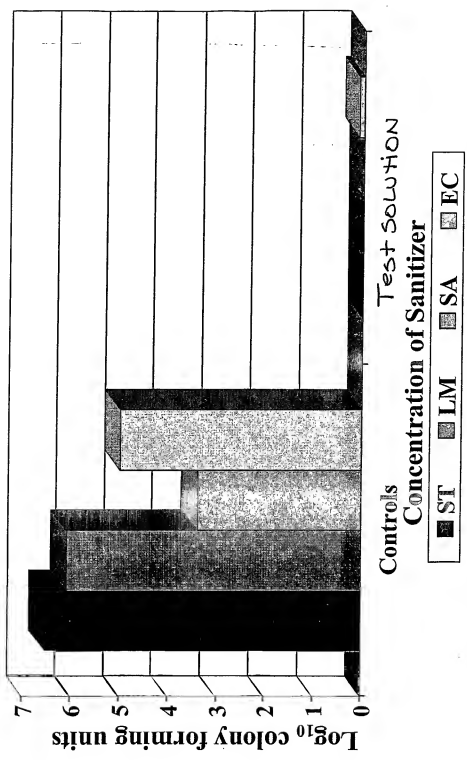
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 5



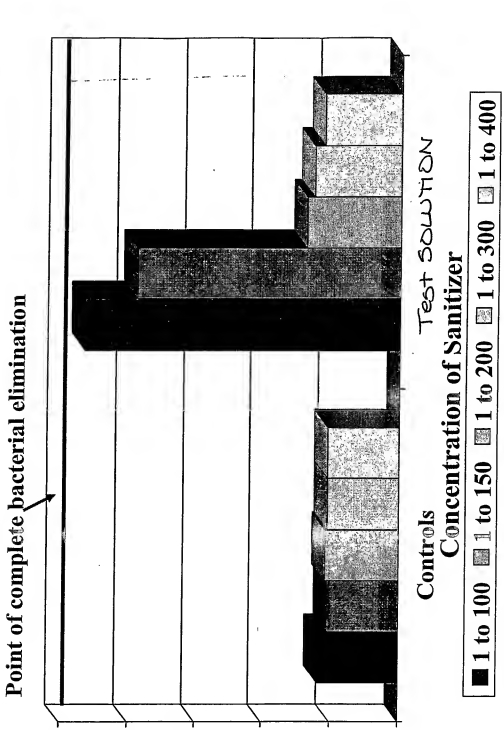
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 6



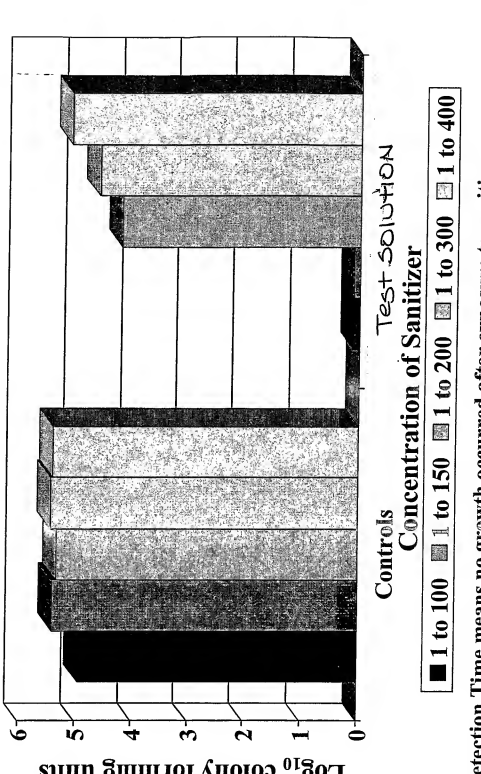
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 7



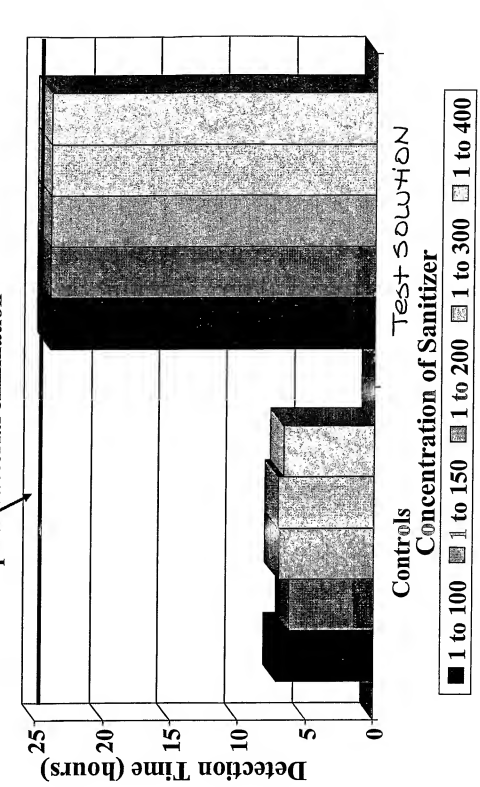
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 8



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 9



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 10

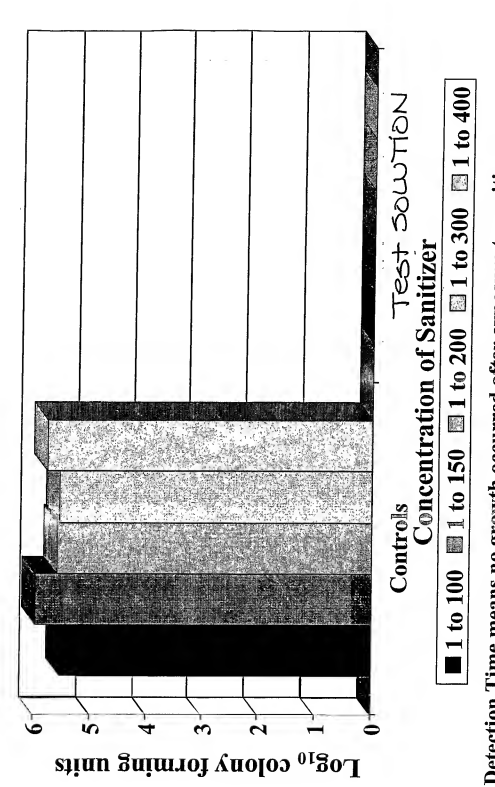

Log10 colony forming units
0
1
2
3
4
5
6
Controls
Test Solution
Concentration of Sanitizer
1 to 100
1 to 150
1 to 200
1 to 300
1 to 400
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 11

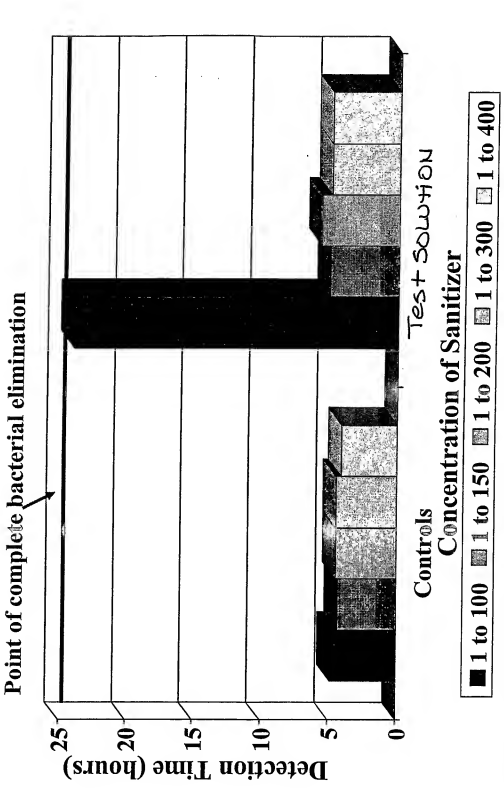
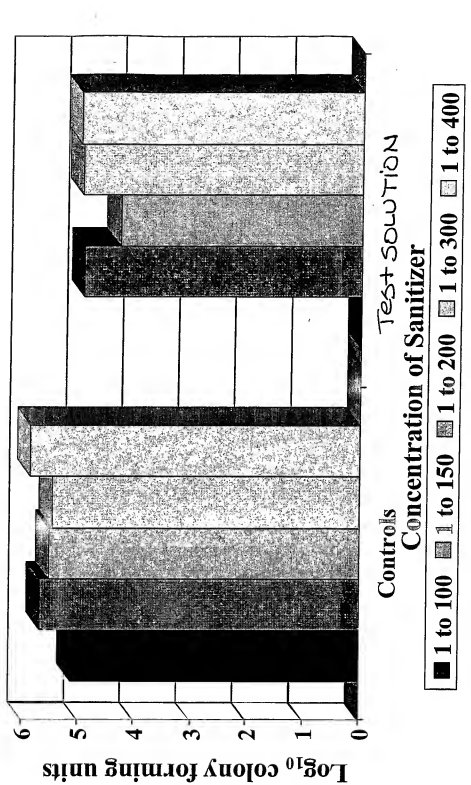
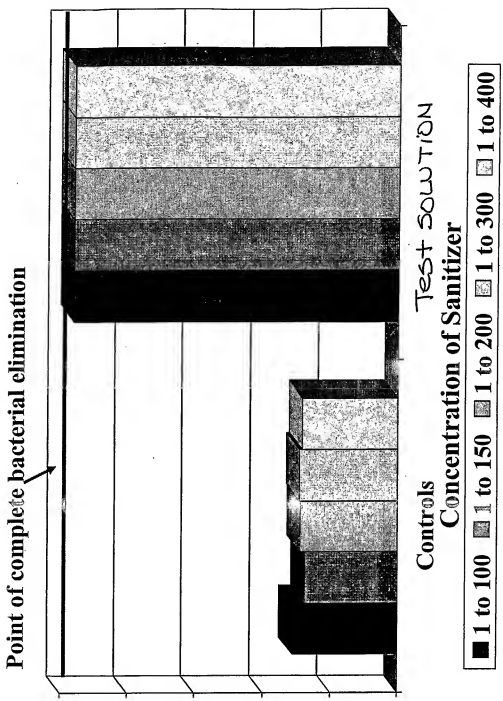

Point of complete bacterial elimination
Detection Time (hours)
25
20
15
10
5
0
Controls
Test Solution
Concentration of Sanitizer
1 to 100
1 to 150
1 to 200
1 to 300
1 to 400
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 12



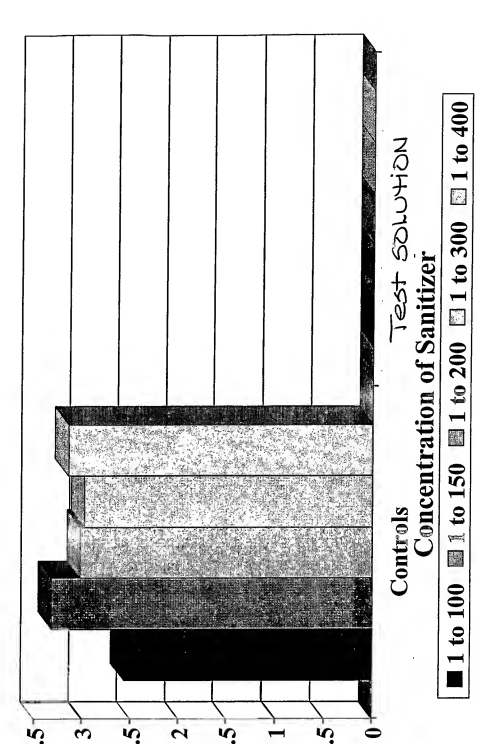
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 13



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 14



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 15

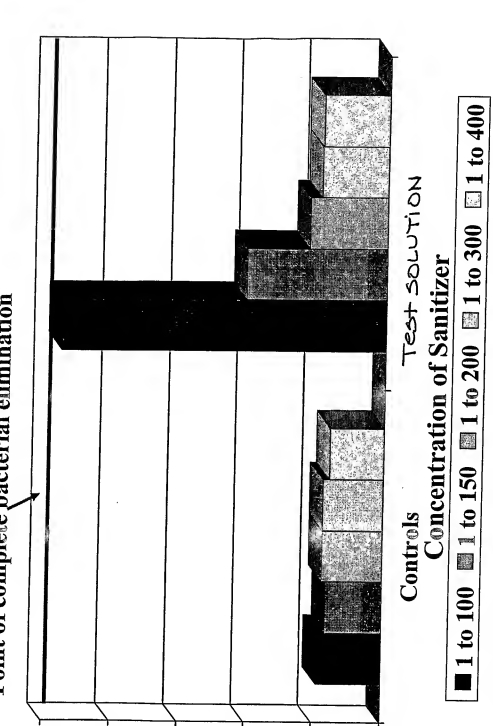
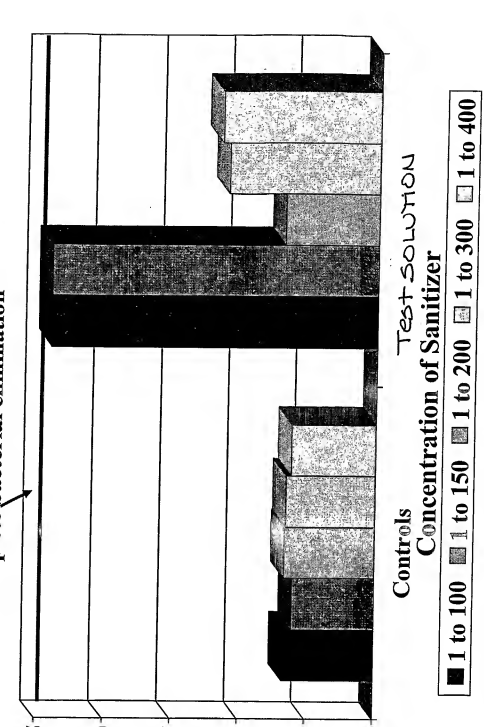

Point of complete bacterial elimination
Detection Time (hours)
25
20
15
10
5
0
Controls
Test Solution
Concentration of Sanitizer
1 to 100
1 to 150
1 to 200
1 to 300
1 to 400
24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 16



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 17

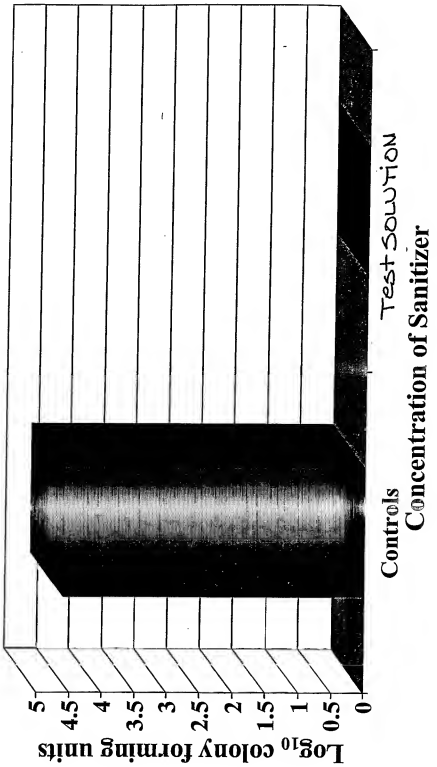
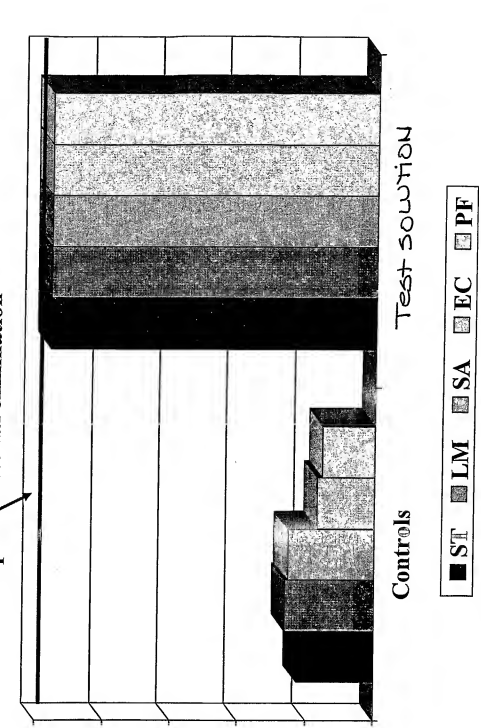

Log10 colony forming units
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Controls
Test Solution
Concentration of Sanitizer

Figure 18



24 hour Detection Time means no growth occurred after exposure to sanitizer

Figure 19

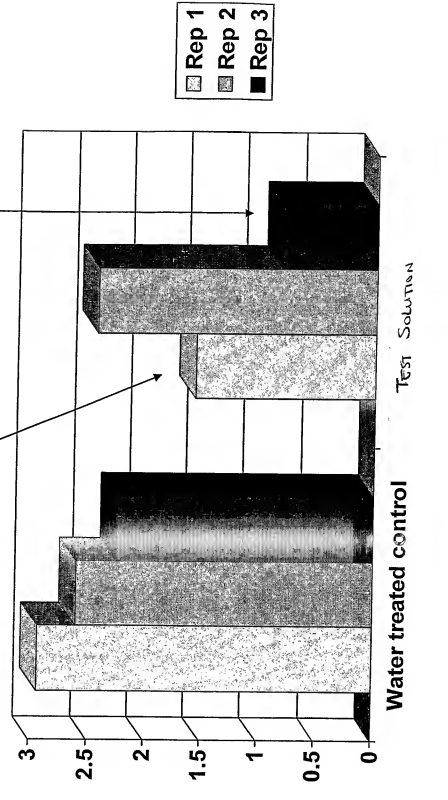
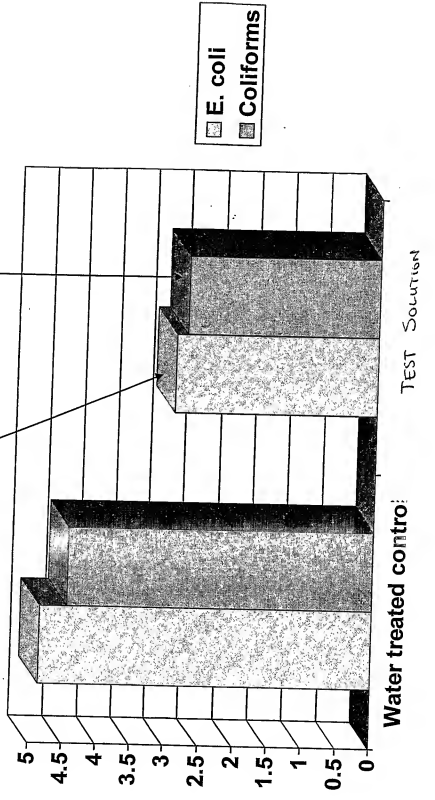

1.4 log10 reduction
53% reduction in
Positive Carcasses
1.4 log10 reduction
60% reduction in
Positive Carcasses
Log10 colony forming units/ml
3
2.5
2
1.5
1
0.5
0
Water treated control
Test Solution
Rep 1
Rep 2
Rep 3

Figure 20


Log10 colony forming units/ml
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1.92 log10 reduction
1.68 log10 reduction
E. coli
Coliforms
Water treated control
TEST SOLUTION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/06599

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A23B 4/24
US CL : 426/331, 335, 532
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 426/331, 335, 532

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,366,983 A (LATTIN et al.) 22 November 1994, abstract.	1-20
Y	US 5,855,940 A (COMPADRE et al.) 05 January 1999, abstract and col. 14, lines 25-30.	1-20
Y	US 6,749,804 A (Schneider et al.) 15 June 2004, abstract and col. 6, lines 45-50.	1-20
Y	US 5,906,825 A (SEABROOK, JR. et al.) 25 May 1999, abstract and col. 7, lines 49-52, and col. 8, lines 54-59.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 July 2004 (13.07.2004)	12 AUG 2004
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Helen F. Pratt Telephone No. 571-272-1201

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 3:
WEST search terms: trichloromelamine, bacteria, food, poultry, quaternary ammonium, trimethyl ammonium chloride, cetypyridium, dimethyl ammonium chloride, didecyl dimethyl ammonium chloride.